

1 GAATTCCAGGCTAGGAAGTGAACCTGGACCCAGCTCACGGGCAGCAG 60
 61 CGGCAGCAGGCAAGCCTCTATCCCTCCAGCCACATGGCCCCGGATGGCGCT 120
 MetGlyProArgMetAlaLeu
 121 CCCCGGGCTCCTGCTCCTGACCTGTGCTAGGATGCCGTTCCCATCCA 180
 ProArgValLeuLeuLeuPheLeuHisLeuLeuLeuGlyCysArgSerHisPro
 eProAlaCysSerCysSerCysSerCysthrCysCysCysEndAspAlaValProIleH1
 erProArgAlaProAlaProValLeuAlaProValAlaAlaArgMetProPheProSerT
 181 CTGGGTGGCCTGGCTCAGAACACTGCCAGGGATAACAGGTGAGGCCCTGATGAACTG 240
 LeuGlyGlyAlaGlyLeuAlaSerGluLeuProGlyIleGlnValSerProAspGluLeu
 sTrpValAlaLeuAlaTrpProGlnAsnCysGlnGlyTyrArgEndAlaLeuMetAsnCy
 hrGlyTrpArgTrpProGlyLeuArgThrAlaArgAspThrGlyGluProEndEndThra
 241 CTTAGACTTGGCTGGGAGGGGGAGCAGCAACTAACGGGTCCCCCACCTACTG 300
 LeuArgLeuGlyTrpLeuGlyGlyArgGlyGlnGlnGlnLeuThrGlyProHisLeuLeu
 sLeuAspLeuValGlyTrpGluGlyAlaAspSerSerAsnEndArgValProThrTyrCy
 laEndThrTrpLeuAlaGlyArgAlaArgThrAlaAlaThrAsnGlySerProProThrV

FIG. 1A

| | | |
|-----|--|-----|
| 301 | TTCCAAGAGGGCTAACCTCCTTGGAAACTAGTGATAAGGGGTTAGAAGGCCAG PheGlnGluGlySerAsnLeuLeuTrpGluLeuValIleArgGlyLeuGluGlySerGln sSerLysArgAlaLeuThrSerPheGlyAsnEndEndGlyValEndLysAlaAlaAr a1ProArgGlyLeuEndProProLeuGlyThrSerAspLysGlyPheArgArgGlnProG | 360 |
| 361 | GCTGGGGGTGAGGACCCGGCTCCCAAGGCAGTTGGCTTCAGCACCATCAAGAGTGTAT AlaGlyGlyGluAspProLeuProArgGlnLeuValArgPheSerThrIleLysSerAsp GLeuGlyValArgThrArgSerGlnGlySerTrpPheAlaSerAlaProSerArgValMe lyTrpGlyEndGlyProAlaProLysAlaValGlySerLeuGlnHisHisGlnGluEndT | 420 |
| 421 | GGTCCAGGGTAGTTCCCTGAGGCTCGGGCTCCCCCACCCATCCAGGAGCTGCTGGAC GlySerArgCysGluPheLeuArgLeuGlyLeuProHisProSerGlnGluLeuLeuAsp tGlyProGlyAlaSerSerEndGlySerGlySerProThrHisProArgSerCysTrpTh rpValGlnValArgValProGluAlaArgAlaProProProIleProGlyAlaAlaGlyP | 480 |
| 481 | CCCCTGGAGACAGGGTCTCCGAGCTGGAGGGACGGACCTGGAGGCCCTCCGGC ArgLeuArgAspArgValSerGluLeuGlnAlaThrGlyArgThrTrpSerProSerGly rAlaCysGluThrGlySerProSerCysArgArgAspGlyProGlyAlaProProAl roProAlaArgGlnGlyLeuArgAlaAlaGlyAspGlyThrAspLeuGluProLeuArg | 540 |
| 541 | AGGACCGTGGCCTCACAGAACGCCCTGGAGGGACGGGAGCCACGGGGCTCTTG ArgThrValAlaSerGlnLysProGlyArgArgGlyLysGlnProProArgGlyPheLeu aGlyProTrpProHisArgSerLeuGlyGlyGluGlySerProHisGlyGlySerTr lInAspArgGlyLeuThrGluAlaTrpGluAlaArgGluAlaAlaProThrGlyValLeuG | 600 |

FIG. 1B

601 GCCCCGGCAGTAGCATCTTCCAAGTCCTCGGGAAATACGGAGCCCCAAGACGATGGTG 660
Gly Pro Ala Val Ala Ser Ser Lys Ser Ser
PAla Pro Gln End His Leu Pro Ser Pro Pro
ly Pro Arg Ser Ser Ile Phe Gln Val Leu Arg Gly Ile Arg Ser Pro Lys Thr Met Arg A

661 ACTCTGGCTGGCTTGGGGAGGGCTGGACCCGGATCGGCCCTCCCTCAGGGGGCCTGGCTGCCA 720
sp Ser Gly Cys Phe Gly Arg Arg Leu Asp Arg Ile Gly Ser Leu Ser Gly Leu Gly Cys A

721 ATGGTGAGCAACCCACCCATTCCCACACTGGCACGGGGTTAGCATCACTTCTGGTTGA 780
snV

781 TGTCTCTGGGACCAAACACTCCGAGAAAAGGACACCTGGATATCACTCTTGTGCCAG 840

841 TCCTCAAGGCCAAGGAGGCCCTCCTGGAAAATTAAATTGGACAGCATTCACTAGCAT 900

901 GACTATGAGTCCCCACCCACCTTCTGGCCACCCCCCTGCCTCTCTCACCCAAAGGGCAGA 960

961 ATTACTTAGGATGTAATTCTGTCAATTGGCTGGCTCCCTGGGAGCAAAAGAGA 1020

FIG. 1C

1021 ACTAACCTCTCCCTGGTTCCCTCAACTGTCCTGGCTGCAAAGGCAGAGGCCAG 1080

1081 GATCACCAGGGTGTGACAAGTCCCAGCTTACAAGGAGGAAACTCAGGTCCAGAGATG 1140

1141 GATTATCCCAGCCCCAACATCCAGTTCTGCTGAAGAAGGGGGTGGCAGGGTGGCA 1200

1201 CGTGGTGGGGAAAGCCCAGGTCCTGGCTCTCACCCCTTAATGTCATCACCCTCT 1260

1261 CTCTCCCCCACAGTGCTCAGGAGGTACTGAGAAGTCCTGGCTGACAACCTCTGTGCC
alleuArgArgTyr** 1320

1321 GCTTCTCCAACGCCCTCCCTGGCTCCCTCAAAGCAACTCCTGTTTATTATGTAT 1380

1381 TTATTATTATTATTGGTGTATAAGACGGTTCTTATTGTGAGCACATTTT 1440

1441 TTCCATGGTGAATAAGTCAACATTAGGCTCTGTTGAAAAAAAAGGA 1500

1501 ATTC 1504

FIG. 1D

BNP Screening Oligos

5' -TCCAGCTGCTTCGGGCCAGGATGGACAGGATTGGAGCCCCAGAGCGGACTGGCTGTAAC-3'
SerSerCysPheGlyGlyArgMetAsPArgIleGlyAlaGlnSerGlyLeuGlyLeuGlyCysAsn-3'
(2)
SerGlyCysPheGlyArgArgLeuAsPArgIleGlySerLeuSerGlyLeuGlyCysAsn
5' -ACNGGNTGCTTGGGNCGNCGNCTNGACCGNATNGGNTCNCTNTGGNCTNNGNTGCAAC-3'
TG T A A T TA AG T AG T T T (minimal)
3' -AGGCCACGAAGCCCCGGTCCGACCTAACCTAGGAACTGGCTGACCCGACATTG-5' 3351 (minimal)
3' -TCGGCCACGAAGCCGGTCTTAGCCGTCTGAGCTGCTTAGCCGTCTGGAGTCCGGGACCGTTG-5' 3352 (G/T pref)
3' -AGGTCCACCAAGCCCCGGTCTAACCTGTCCATAACCTGGCTCTCCCTGACCCGACATTG-5' 3376 (ANP)

FIG.2

hn BNP cDNA (10-13-88)

1 GAATTCCAGGCTTAGGAAGTGAACCTGGACCCAGCTCAGGGCAGGCAGGGCAGCAG 70
71 CAGCAGGCCACATGGGCCCTGGATGCCCTTCCCCCGTGTCTGGCTCCTGGCTCCT 140
MetGlyProArgMetAlaLeuProArgValLeuLeuLeuLeuLeuLeuLeuLeu
141 GTTCTTGACCTGCTGCTAGGATGCCGTTCCCATCCACTGGCTGCCCTGGCCTCAGAACTG 210
uPheLeuHisLeuLeuLeuLeuLeuLeuLeuLeuLeuLeuLeuLeuLeuLeuLeu
10 -1 +1 10
211 CCAGGGATAACAGGTGAGCCCTGATGAACCTGGCTGGCTGGACTTGGACTTGGAACT 280
ProGlyIleGln
281 TAACGGGTCCCCACCTACTGTTCCAAGAGGGCTCTAACCTCCTTGGAACTACTAGTGATAAGGGGTAGAA 350
351 GGCAGCCAGGCTGGGGTGAAGGACCCGGCTCCAAAGGCAGTTGGCTTCAGCACCATCAAGAGTGAT 420
421 GGTTCCAGGTGGAGTTCCGTGAGGCTGGCTGGCTGGGCTGGGACCCATCCCAAGGAGCTGGTGGACCGCCCTGGGAG 490
GluLeuLeuAspArgLeuArgA
20.
56C
491 ACAGGGGTCTCCGAGCTGCAGGGACGGACCTGGAGGCCCTCCGGCAGGACCCCTCACAGA 56C
spArgValSerGluLeuGlnAlaGluAspLeuArgThrAspLeuArgGlnAspArgGlyLeuThrGln
40.
561 AGCCCTGGGAGGGCAAGCAGGCCCTGGGGTCTGGCCCGCAGTAGGCATCTTCCAAAGTCCTC 63C
uAlaTrpGluAlaArgGluAlaAlaProThrGlyValLeuGlyProArgSerSerIlePheGlnValLeu

FIG.3A

50. → ← 60. → ← 70.

| | |
|---|------|
| 631 CGGGGAATACGGCAGCCCCAAGACGATTCGGCTGACTCTGGCTTGGCGGAGGGCTGGACCGGATGGCT | 700 |
| Arg Gly Ile Arg Ser Pro Lys Thr Met Arg Asp Ser Gly Cys Phe Gly Arg Arg Leu Asp Arg Ile Gly S | |
| 80. ↑2 | 90. |
| 701 CCTCAGGGCCTGGCTGCAATGGTGAGCACCCACCCCCATTCCCACACTGCACGCCGGTTAGCATCAC | 770 |
| Leu Ser Gly Leu Gly Cys Asn V | |
| 100 | |
| 771 TTCTGGCTTGTATGGTCTCTGGGGACCAAACTCCGAGAAAAGGACACCTGGATATCACTCTTCTTGTGCC | 840 |
| 841 CAGTCCTCAAGGCCAAGGAGGCCCTTCCTGGAAAATTAAATTGGACAGGCATTCACTAGCATGACTATG | 910 |
| 911 AGTCCCCACCCACCTTCTCGCCACCCCCCTGCCTCTCACCCAAAGGGGGCAGAACATTACTTAGGATGTAA | 980 |
| 981 ATTCTGTCAATTGGCTCTGGAGCAAAGAGAACTAAACCTCTCCCCCTGGTTCCCC | 1050 |
| 1051 TCAACTGTCTGGCTGCAAAAGGCCAGGGCAGGGCAGGGATACCAGGGTGTATGACAAGTCCCAGCTTACAAGGA | 1120 |
| 1121 GGAAAACTCAGGTCCAGAGAGATGGATTATCCCAAAGCCCCAACATCCAGTTCTGCTGAAGAACGGGGT | 1190 |
| 1191 GGCAAGGGTGGCACGGTGGGGGAAGCCCAGGTCCCTGCCTCACCTTAATGTCATCCTCACCC | 1260 |
| 1261 TCTCTCTCCCCCACAGTGCTCAGGGAGGTACTGAGAAAGTCCTGGCTGACAACCTCTGTCCGGCTCTC al Leu Arg Arg Tyr *** | 1330 |
| 1331 CAACGGCCCTCCCTGCTCCCTCAAGCAAACCTCCTGTTTATTATTATTATTATTATT | 1400 |
| 1401 TGGTGGTTGTATAAGACGGTTCTTATTGTGAGCACATTTCATGGTGAATAAGTCAACATTA | 1470 |
| 1471 GAGCTCTGCTTITGAAAAAAAGGAATT C 1507 | |

FIG.3B

Mature Pig BNP cDNA (10-13-88)

FIG. 4

Dog BNP Gene 12-12-88

1 CGATCAGGGATGTTGGGGAGGAACGGAGGGAAACGGAGGGAGGGAGGGACTGGTGGTG 70
71 TCCCCCTCCTGGCCCACTTCTATAAGGCCTGCTCTCCAGCTCCACCCGGCG 140
141 GGTATGGTGCAGGGGGGGCATTCGGCTGAGCTCAGGGGGGAATGGGGCCGATAAAT 210
211 CAGAGATAACCCAGGGCGGGATAAAGGGATAAAAAGCCCCGTGGGGATCCAGGAGAGCACCCG 280
281 CGCCCCAAGGGGTGACACTCGAACCCGGTGGCAGGGAGCTCAGGCCGACGTCTTCCCCAC 350
351 TTCTCTCAGGGACATGGAGGCCCTGGCCAGGGCTGGCCCTCTGGTCTTGCACCT 420
Met Glu Pro Cys Ala Ala Leu Pro Arg Ala Leu Leu Phe Leu His Le
421 GTGCCCACTCGAGGCCCTGGCCAGGGCTGGCCCTCGGAAGGCCCTCGGAAGGCCCTCA 490
uSer Pro Leu Gly Gly Arg Pro His Proleu Gly Arg Ser Pro Ala Ser Glu Ala Ser
491 GAAGCCTCGGGTGTGGCCGTCAGGGTGAAGCTCAGGCCCTGAAGGCCGGGGTGGCAGCAG 560
561 GTCACGGGGCTTAGCCCAAGTCTCAGTCTCCCTGGAAATTAGTGATAAGGGAATCAGAAA 630
631 GTGACCGAGATTGGTGCCAGGACTCCATACCCAAGGGGGCTCACTTGGTGTGCAAGGGTGGTCCGC 700
701 CCCGGCCTGGGTCTGGCTCAGGCCCTGAGGACTGAGGAGCTGCTGGCCCTCTGAAGGACGCAGTGT 770
Glu Leu Gly Arg Leu Lys Asp Ala Val S

FIG.5A

| | | |
|------|--|------|
| 771 | CAGAGCTGCAGGCCAGGCAACTTGGCCCTTGAACCCCTGCACCGGAGCCACAGCCCCGCAAGCCCCGGAA erGluLeuGlnAlaGluGlnLeuAlaLeuGluProLeuHisArgSerHisSerProAlaGluAlaProG1 | 840 |
| 841 | GGCGGGAGGAACGCCCCGGTCCCTTGACACCCATGACAGTGTCTCCAGGCCCTGAGAAGACTACGCC uAlaGlyGlyThrProArgGlyValLeuAlaProHisAspSerValLeuGlnAlaLeuArgArgLeuArg | 910 |
| 911 | AGCCCCAAAGATGCAAGTCAGGGTGTCTGGCCGGAGGCTGGACCGGATCGGCTCCCTCAGTGGCC SerProLysMetThrProHisLysSerGlyCysPheGlyArgArgLeuAspArgIleGlySerLeuSerGlyL | 980 |
| 981 | TGGGCTGCAATGCTTAAGCCGGCTTCCCTGGCCCTTGGCTCCCCCTGGCCCTTGGCTCCCTGGACCCCTT euGlyCysAsnV | 1050 |
| 1051 | GGAAACCCCTTCTGGTTTGTCTCGGGATCACACTCTGAGGAAGAACATCTGGACATCTGCTCCTT . | 1120 |
| 1121 | CTTGGCTGACAGTCTTAAGGGCCAAGGAGTACGTTCTGGAAATACTACGTTGACATCGTTGTCAGGG . | 1190 |
| 1191 | TCCCTACCCACCTCTAGCCCCCTGCTCTGGCACCCAAAGGCAGAAATCATCTTAGCATGGAATCA . | 1260 |
| 1261 | GTCGTTGTCGAAGGATCTCCTGGAGCAGAAAGAGTCCTAAACATCGTCCTCGTAGCTCTCTGTCT . | 1330 |
| 1331 | GTCCTGACCGAAGGAGGGTCAAGGGCACCCAGGGCAGTGTGATTCCCAAGTAAACAGGAGGAGA . | 1400 |
| 1401 | CTGAGGTCTAGAGAGATGGATTATTCCAAGCCTCAAACATCCAGATCGGCTGAGGGTGGTGGC . | 1470 |
| 1471 | AGGGATGGCTCCTGGCTTGGGAAGCTGGCTCAGTCTCCACCTGACGCCATCATCCCCCTC . | 1440 |
| 1541 | TCTCTCCCACAGTGTGAGAAAGTATTAAAGGAGGAAGTCCCGACTGCCCACATCTGGATTCT . | 1610 |

FIG. 5B

20 30 40 50 60 70 80

alleuArglysTyr***

1611 TCACCAACCCTAACACCACATCTTATTCCATTATTTTCATTGATTG
1681 TTATATAGATGATCCTGGACCGATTCACGGATCTGCATTAAACTACATCATTAAAAA 1804
1751 CTTTCAACCCATTGTCCTGTGCAATTAAAGTACACATCATTAAAAA 1880

FIG. 5C

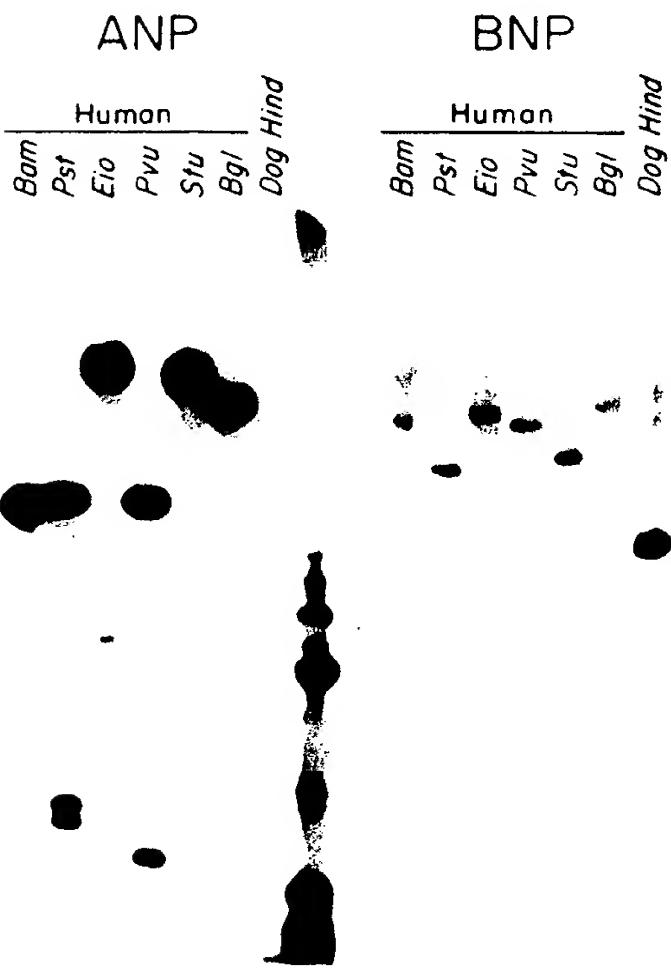


FIG.6

Human BNP Gene 12-12-88

FIG. 7A

| | | |
|------|---|------|
| 701 | TTTGGGAGGAAGATGGACCGGATCAGCTCCAGTGGCTGGCAAAAGGTAAAGCACCCCTGCCAC PheGlyArgLysMetAspArgIleSerSerSerSerGlyLeuGlyCysIysV | 770 |
| 771 | CCCGCCCCCTCCCATTCAGTGTGACACTGTAGGTCACTTGGGTTCTCTGGAA | 840 |
| 841 | CCACACTCTTGAGAAAAGGTACACCTGGACATCGCTTCCCTGTAAACAGCCTCAGGGCCAAGGGTG | 910 |
| 911 | CCTTGTGGAATTAGTAATGTGGCTTATTTCATTACCATGGCCCACAATAACCTTCTCCACCTCCTAC | 980 |
| 981 | TTCTTATCAAAGGGCAGAAATCTCCTTGGGTCTGTTATCATTTGGCAGCCCCCAGTGGTCAGAA | 1050 |
| 1051 | AGAGAACCAAAACATTTCCTCCTGGTTAAACTGCTCTATAGTCTCAAAGGCAGAGGCCAGGATCAC | 1120 |
| 1121 | CAGGCAATGATAATCCCCAATTACAGATGAGGAAACTGAGGCTCAGAGACTTGCATTAAAGCCTCAAAC | 1190 |
| 1191 | GCTGTGACTAACAGGGTGGTGGCACACCGATGAGTAAGCTCAGGCCCTGCCTCCATCTCCACC | 1260 |
| 1261 | CTAACCATCATCACCCCTCTCTTCCCTGACAGTGGTGAAGGGCCATTAAAGAGGAAGTCCTGGCTGCAG alleuArgArgHis*** | 1330 |
| 1331 | ACACCTGCTTCTGATTCCACAAGGGCTTCTCAACCCCTGCCCCCTCATCTTCCATTGGAAATTAG | 1400 |
| 1401 | TGATAAAGGAATCAGAAAATGGAGAGACTGGGTGCCCTGACCCCTGTAACCCAAAGGCAGTCGGTCACTTGG | 1470 |
| 1471 | GTGCCATGAGGGCTGGTGAAGCCAGGGCTGGCTCCCTGAGGCTTTA | 1519 |

FIG.7B

Pig PreproBNF
Dog PreproNRP
Human PreproNRP

FIG. 8